AMENDMENTS TO THE SPECIFICATION

Please replace the first paragraph on page 12 of the Specification under the header "BRIEF DESCRIPTION OF THE FIGURES" with the following paragraph:

Figure 1 is a representation showing that *mgr* genomic locus encodes two distinct isoforms. (A) Alignment of the predicted NH_2-terminal amino acid sequence of the p70 isoform of MGR (SEQ ID NO: 12, amino acids 1-128) and BOM (SEQ ID NO: 14, amino acids 1-130). Amino acid identity is denoted by shared upper case letters and similarity by the (+) symbol. Amino acid segments greater than 4 amino acids in length in the consensus sequence are represented by SEQ ID NO: 12, amino acids 26-35 and amino acids 37-72). The first amino acids shared between p61 MGR arid p70 MGR are given in bold. (B) Structure of the human and murine *mgr* genomic loci. Human genomic sequence was downloaded from the GenBank database (Accession Number AC010969) and aligned with cDNA sequences. Murine genomic clones were obtained from a 129 library and mapped by Southern analysis and PCR. Exons are denoted as E1-8 in human and E1-9 in murine. The two human MGR isoforms are denoted as p70 and p49 MGR and the two murine isoforms as p70 and p61 MGR. The scale of 1 kb is shown. (C) Identification of the murine p61 MGR promoter (SEQ ID NO: 47). Sequence was obtained from intron three from the MGR genomic locus and analyzed using the weight matrices of Bucher, *J. Mol. Biol.* 212: 563-578, 1990. The CAP site, TATA box and GC box are indicated. The cDNA start site is shown in arrows, the first ATG is given in bold and the splice site at the end of the first exon of p61 MGR is indicated.

Please replace the paragraph spanning pages 12-13 of the Specification under the header "BRIEF DESCRIPTION OF THE FIGURES" with the following paragraph:

Figure 3 are representations showing that p70 MGR binds to and transactivates the human En-1 promoter. (A) Identifcation of a *grh* consensus DNA binding site in the human En-1 promoter. The consensus sequence for *grh* DNA binding (SEQ ID NO: 50) compiled from an alignment of the *Drosophila Ultrabithorax*, *Dopa decarboxylase* and *fushi tarazu* promoters was compared with the sequence of the proximal human En-1 promoter (SEQ ID NO: 49) and the *Drosophila* engrailed promoter (SEQ ID NO: 48). The closed bracket indicates the extend of the grainyhead binding site in the engrailed promoter as defined by DNAseI footprinting. (B) Human p70 MGR binds to the human En-1 promoter. Nuclear extract from the JEG-3 cell line was studied in an

EMSA with a *Ddc* promoter probe in the presence of pre-immune sera (lane 1), anti-MGR specific antisera (detailed in legend to Figure 2) (lane 2) or cold competitor DNA (lanes 3-5). A 50-fold excess of the *Ddc* probe was used in lane 3 and a 10- and 20-fold excess of a human En-1 promoter probe in lanes 4 and 5, respectively. The migration of the MGR/DNA complex is shown by arrows. (C) Human p70 MGR transactivates the En-1 promoter. COS cells were transiently transfected with the proximal En-1 promoter containing the MGR binding site linked to a minimal γ-globin promoter and a firefly luciferase reporter gene (solid columns), the minimal γ-globin promoter/luciferase reporter gene (open columns) and the TK promoter linked to the Renilla luciferase reporter gene (hatched columns) in the presence and absence of a p70 MGR expression vector (PCI-p70 MGR) as indicated. Transfection with the empty vector (pCI) served as the control. Luciferase levels were corrected for protein concentration and values were derived from two independent experiments performed in triplicate.

Please replace the paragraph spanning pages 14-15 of the Specification under the header "BRIEF DESCRIPTION OF THE FIGURES" with the following paragraph:

Figure 5 is a representation showing the generation of a null allele of GRHL-3. **(A)** Gene-targeting strategy applied to the mouse GRHL-3 locus. The homologous recombination event deleted 2.2 kb of genomic DNA, including the region encoding the entire transcriptional activation domain of the protein. This was replaced with a promoter-less lacZ.polyA cassette fused to the second codon of exon 2 and a Neo^R gene linked to a PGK promoter and flanked by loxP sites. The thymidine kinase gene driven off the MC1-promoter completed the targeting vector. The location of the 5' and 3' probes used for Southern blot analysis of the targeted allele and the size of the expected hybridization fragments prior to excision of the Neo^R cassette are shown. The Neo^R cassette was excised by crossing mice heterozygous for the targeted allele with a kansgenic line expressing the Cre recombinase gene driven off a CMV-promoter. LacZ.polyA, the lacZ gene linked to the rabbit β-globin polyadenylation signal; B, BamHI; S. SpeI. **(B)** Southern blot analysis of two targeted ES cell clones (C7 and B12) and the parental ES cells (G7) with the 5' flanking probe demonstrating site-specific integration by homologous recombination. The size of DNA standards (in kb) is indicated. **(C)** Germ-line transmission of the targeted allele from cell line C7. Southern blotting was performed with the 3' flanking probe on tail DNA isolated from weaned progeny of GRHL-3+/- intercrosses. The size of DNA standards (in kb) is

indicated. **(D)** PCR genotyping of embryos. Two allele, three primer PCR was performed on genomic DNA from E18.5 embryos isolated from GRHL-3+/- intercrosses. The size of DNA standards (in bp) is indicated target, PCR product diagnostic of targeted 25 GRHL-3 allele; wt. PCR product diagnostic of wild type GRHL-3 allele. ~~**(E)** Northern blot analysis of GRHL-3 mRNA expression in wild-type embryos and embryos heterozygous or homozygous for the targeted GRHL-3 allele (upper panel). RNA integrity was confirmed with a GAPDH probe (lower panel). The size of RNA standards is indicated, as is the migration of the GRHL-3 and GAPDH transcripts. **(F)** RT-PCR of E9.5 GRHL-$3^{-/-}$ and GRtIL-$3^{+/-}$ embryos was performed with primers specific for HPRT. Based on the HPRT quantitation, comparable amounts of cDNA from each embryo were PCR amplified for 30, 32 and 35 cycles (GRHL-$3^{+/-}$) or 35, 38 and 40 cycles (GRHL-$3^{-/-}$) with primers specific for GRHL-3. The 5' primer anneals to exon 8 and the 3' primer anneals to exon 13. Both primer pairs gave predicted size bands of 503 bp for GRHL-3 and 229 bp for HPRT. The identities of the amplified bands were confirmed by Southern blotting using gene specific internal oligonucleotides.~~

Please replace the paragraph spanning pages 14-15 of the Specification under the header "BRIEF DESCRIPTION OF THE FIGURES" with the following paragraph:

Figure 7 is a representation showing GRHL-3 and ct are the same gene. **(A)** Organization 20 of the ct candidate region. Genetic map of the 13 Mb supercontig (Accession number NW_000213) that shows the positions of relevant markers (D4Mit69 and D4Mit157) and previously excluded ct candidate genes (*Synd3*, *Fgr*, *Hspg2*, *Pax7*). The position of the GRHL-3 locus is also indicated. The size of the interval between the GRHL-3 locus and the D4Mit69 marker is shown. **(B)** Morphological appearance and genotype of embryos derived from ct/ct mice crossed with GRHL-$3^{+/-}$ mice. Embryo 1 is unremarkable; embryos 2 and 3 display curly tails (arrowheads); embryos 4 and 5 display curly tails and lumbo-sacral spine bifida (arrows). ct. curly tail; SB, spine bifida. Scale bar = 10mm. **(C)** Total RNA from E14.5 embryos from *curly tail* (*ct/ct*), wild type (+/+) and GRHL-3 heterozygotes (+/-) were analysed for GRHL-3 expression by Northern blotting with a cDNA probe derived from the unique coding portion of the mRNA described in Fig 1A (upper panel). RNA loading was monitored by probing with 28S (lower panel). Signal intensity was quantified by Phosphorimager densitometry and the individual embryo GRHL-3 signals corrected for 28S loading. The corrected signal intensities relative to wild type embryo 7

are shown. Positions of GRHL-3, 28S and the RNA size standards are indicated. **(D)** Quantitative real-time RT-PCR was performed on total RNA from E14.5 5 *curly tail* (*ct/ct*), wild type (+/+) and GRHL-3 heterozygous (+/-) embryos. A standard curve was generated for HPRT and GRHL-3 and the relative quantity of both transcripts was calculated for individual embryos. Each reaction was performed in duplicate. The ratios of GRHL-3/HPRT in GRHL-$3^{+/-}$ and *ct/ct* embryos were normalised to the values obtained with GRHL-$3^{+/+}$ embryos. **(E)** Northern blot analysis of GRHL-3 mRNA expression in wild type embryos and embryos heterozygous or homozygous for the targeted GRHL-3 allele (upper panel). RNA integrity was confirmed with a GAPDH probe (lower panel). The size of RNA standards is indicated, as is the migration of the GRHL-3 and GAPDH transcripts. **(F)** RT-PCR of E9.5 GRHL-$3^{-/-}$ and GRtIL-$3^{+/-}$ embryos was performed with primers specific for HPRT. Based on the HPRT quantitation, comparable amounts of cDNA from each embryo were PCR amplified for 30, 32 and 35 cycles (GRHL-$3^{+/-}$) or 35, 38 and 40 cycles (GRHL-$3^{-/-}$) with primers specific for GRHL-3. The 5' primer anneals to exon 8 and the 3' primer anneals to exon 13. Both primer pairs gave predicted size bands of 503 bp for GRHL-3 and 229 bp for HPRT. The identities of the amplified bands were confirmed by Southern blotting using gene-specific internal oligonucleotides.

Please replace the paragraph 78 of the Specification under the header "EXAMPLE 16" with the following paragraph:

To determine the functional role of GRHL-3 during mouse development, a 2.2 kb deletion in GRHL-3 was generated by gene targeting (Fig. 5A-D). Northern blot and RT-PCR analysis indicated that the targeted GRHL-3 allele represented a null mutation (Fig. 7E and F~~5E,F~~). Genotyping of offspring from GRHL-$3^{+/-}$ intercrosses from mid and late gestation, showed that GRHL-$3^{-/-}$ mice were represented in Mendelian proportions up to E18.5. Of 874 embryos examined on, or before this time, 191 (22%) were genotyped as GRHL-$3^{-/-}$. No GRHL-$3^{-/-}$ embryos survived to weaning.